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TRP Channels I

613-Pos Board B393
A Mechanism for Detecting the Rate of Temperature Change in Drosophila
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Temperature sensation is crucial for an animal's growth and survival. In addition to detecting actual temperatures, animals are also sensitive to the rate of temperature change. Many animals exhibit a nociceptive response only if the rate of temperature change is rapid. However, the molecular mechanisms through which animals are able to sense the rate of temperature change are unknown. To tackle this question, we focused on Drosophila larvae. We built a Peltier-based temperature control system to precisely control the rate of temperature change and used customized programs to automatically track and analyze larval rolling behaviors. We found that the nociceptive behavior (rolling) was triggered by a high temperature change rate, but not high temperatures themselves if the rate of temperature change was low. To identify a candidate molecular sensor that might contribute to sensing the rate of temperature change, we tested contributions of thermosensitive Transient Receptor Potential channels (thermoTRPs), since these channels play important roles in temperature sensation in organisms ranging from worms to humans. We found that the TRPA1 and a subset of TRPA1-expressing neurons in the larval brain are required for sensing the rate of temperature change. We propose that larvae use the rate of temperature change as an ''alert'' signal to trigger rolling behaviors, allowing a fast escape from a deleterious thermal landscapes before the temperature rises to dangerous levels.

614-Pos Board B394
Electrophysiological Characterization of Internal and External Ligands on TRPA1
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TRPA1 is an ion channel expressed on peripheral and spinal sensory neurons and it mediates pain signal transmission. It functions as a cellular sensor for detecting painful mechanical, biochemical and thermal stimuli that cause sensory nerve hyperactivity during chronic pathologies including chronic pain, inflammation, itch and cough. TRPA1 receptor is shown to induce pain hypersensitivity in animal models of diabetic neuropathic pain and its blockade attenuates pain hypersensitivity as well as later loss of the nerve fibers and their function.
The Results show different methods of stimulating TRPA1 in high throughput patch clamp experiments with the SyncroPatch 384PE.

615-Pos Board B395
Cooling Down TRP Channel: Cold and Chemo-Sensitivity of the Human TRPA1
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Transient receptor potential (TRP) channels are an important class of receptors found widely distributed throughout the mammalian central and peripheral nervous systems. They have been shown to be activated and regulated by a variety of stimuli including temperature, mechano-stimulation and different kinds of molecules, which mediate the sensation of taste, as well as divalent cations and pH. Dysfunction of TRP channel can cause various pathological conditions, including an inherited pain syndrome, multiple kidney diseases and skeletal disorders. Hence, TRP channel become potential targets for the treatment of such disorders.
Patch clamp electrophysiology remains the gold standard for studying ion channels. We have employed a planar patch clamp technology to study the purified human TRPA1 channel (hTRPA1). Solvent-free planar lipid bilayers can be formed in an automated fashion by positioning and subsequent bursting of giant unilamellar lipid vesicles containing ion channels, here hTRPA1, on the Port-a-Patch micron-sized apertures borosilicate glass substrate.
In this study, the human TRPA1 was purified and reconstituted into lipid bilayers and single channel currents were recorded to understand the thermo- and chemosensory properties of the channel together with the role of the N-terminal ankyrin repeat domain (ARD). We report that hTRPA1with and without its N-terminal ARD is intrinsically cold-sensitive, and thus cold sensing properties of hTRPA1 reside outside the N-terminal ARD.
Furthermore, hTRPA1 is activated by a range of environmental irritants, pungent compounds found in foods such as garlic, mustard and cinnamon, as well as metabolites produced during oxidative stress. Data will be presented showing activation and inhibition of the hTRPA1 channel reconstituted in bilayers as well as hTRPA1 expressed in HEK cells (Millipore), using the SyncroPatch 384PE planar patch clamp platform, by a variety of agonists and antagonists.

616-Pos Board B396
The Role of TRPA1 and TRPV1 Channels in Orofacial Pain
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Inflammation of the trigeminal nerve is considered one of the most painful conditions known to humankind. The diagnosis is often difficult moreover, safe and effective pharmacological treatments are lacking.
Transient receptor potential (TRP) channels are a large family of non-selective cation channels. Several TRP channel family members, including TRP cation channel subfamily V member 1 (TRPV1), subfamily A member 1 (TRPA1) and TRP channel melastatin 8 (TRPM8), are expressed in TG somatosensory neurons, which also project within the oral and nasal cavities and are deputed to detect a great deal of external stimuli, including pressure, temperature, and chemicals.
In recent years many efforts have been dedicated to the discovery of better and safer analgesics. However the medical need for this type of drugs remains substantially unmet. In particular, compounds capable of targeting both inflammatory and neuropathic pain are lacking.
Thus, capitalizing on recent results [1] about the ability of the synthetic TRPA1 antagonist ADM_09 to revert oxaliplatin-induced neuropatic pain, and given the evident role of TRP channels in TG-related pain, we report herein on the synthesis of a new lipoic-containing antagonist namely ADM_12. This water-soluble small molecule, structurally simpler and more stable than ADM_09, showed: i) a remarkable safe profile; ii) a high binding constant vs. TRPA1; iii) an intriguing behaviour vs. TRPV1 and iv) the ability to significantly and persistently reduce mechanical facial allodynia in rats. Noteworthy, testing ADM_12 we shed light on the unprecedented involvement of both TRPA1 and TRPV1 channels in orofacial pain.
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617-Pos Board B397
Probing Temperature Sensing of Thermal TRP Channels by Calorimetry
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Proteins such as ion channels generally involve specialized mechanisms for optimal interaction with stimulus. Thermal TRP channels are molecular entities in mammals for detection and transduction of temperature among other pain-evoking stimuli. Sensitive detection of temperature by thermal TRP channels is made possible by large enthalpy changes between closed and open states of the channels. Thus, to understand the mechanism of their temperature sensitivity and to properly identify the underlying molecular and structural basis, it is important to have the capability to directly probe the energetics of temperature sensing by these channels. Unfortunately, direct measurement of the energetics of the channels has not been possible; instead they are inferred from functional measurements, typically based on the steepness of temperature dependence of current responses. Since thermal TRP channels are allosteric proteins and the gating convolves multiple steps, such measurements are indirect and have limitations. In this work we tested the idea of using calorimetry to directly probe the energetics of thermal TRP channels. Modern colorimeters have a sensitivity in the range of micro calories, and our experiments suggest that the energetics of thermal TRP channels are within this detectable range. Thus our results support the applicability of calorimetry for direct detection of thermal transitions in thermal TRP channels,